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Previews

iNKTs Foil Fungi

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Fungal cell wall polysaccharides are potent inducers of immune responses. Cohen et al. (2011) demonstrate that innate recognition of fungal β-(1,3) glucan triggers effector functions of invariant natural killer T (iNKT) cells through indirect, cytokine-driven activation, a process that mediates optimal protection against the opportunistic mold Aspergillus fumigatus.

Human pathogenic fungi, a small subset of the ~10^5 known yeasts and molds in the fungal kingdom, have emerged as increasingly frequent causes of infectious morbidity and mortality, largely due to the AIDS pandemic and advances in medical technology, such as indwelling catheters, chemotherapy, transplantation, and targeted immunosuppression. *Aspergillus fumigatus,* a saprophytic mold, causes allergenic disease in asthmatic and cystic fibrosis patients, and invasive disease in immune-compromised patients, particularly in those with leukemia and in hematopoietic stem-cell transplant recipients. Humans inhale several hundred conidia (spores) daily. Typically, this encounter is symptomless and characterized by rapid fungal clearance with a primary role for neutrophils and alveolar macrophages. In this issue of *Cell Host and Microbe*, Brenner and colleagues report that natural killer T (NKT) cell-deficient mice are defective in clearing a respiratory *A. fumigatus* challenge (Cohen et al., 2011).

NKT cells are a heterogeneous population and part of the evolutionarily conserved family of innate-like T lymphocytes. The predominant NKT cell population recognizes lipids in the context of the nonclassical MHC molecule CD1d. These NKT cells typically express a T cell receptor (TCR) composed of an invariant α chain (with a Vα14-Jα18 or a Vα24-Jα18 rearrangement in mice and humans, respectively) paired with a limited set of β chains. Functionally, these iNKT cells have the capacity to release vast quantities of diverse effector cytokines, including IFN-γ, IL-4, and IL-17, within minutes of primary activation, facilitated in part by preformed stores of cytokine transcripts.

How iNKT cell activation is coupled to microbial recognition has been a focus of intense interest. In bacterial, parasitic, and viral infections, two principal modes of iNKT cell activation have been established (Tupin et al., 2007). Direct activation can occur when CD1d presents microbial lipid antigen to the iNKT TCR. Although first demonstrated for the marine sponge artificial antigen α-galactosylceramide, subsequent studies have shown that α-proteobacterial glycosphingolipids and galactosyldiacylglycerols lead to iNKT cell activation. Similarly, highly virulent bacteria, exemplified by *Streptococcus pneumonia*, a leading cause of pneumonia and meningitis, contain glycolipid antigens (α-glucosyldiacylglycerols) that, when presented on CD1d, stimulate iNKT cell activation in vivo (Kinjo et al., 2011). Direct activation is not restricted to bacteria, as lipids from the parasite *Leishmania donovani* can directly activate iNKT cells in a CD1d-dependent, IL-12-independent manner (Tupin et al., 2007).

Indirect iNKT activation occurs when microbes induce innate cytokine production in a Toll-like receptor (TLR) signaling-dependent process and iNKT cells are in turn activated by these cytokines. This type of activation occurs after systemic *Salmonella typhimurium* infection, typically in conjunction with CD1d-dependent presentation of an endogenous lipid. A recent study provides insight into how iNKT cells can recognize both α-linked foreign and β-linked endogenous lipids in the context of CD1d (Pellicci et al., 2011). How specific conditions dictate which endogenous lipids are presented on CD1d remains poorly understood. Viruses use host lipids as the sole lipid source and activate iNKT cells in an indirect manner. Presentation of endogenous lipids by CD1d typically plays a minor role in this type of indirect activation (Tyznik et al., 2008).

The two activation modes are not mutually exclusive, and some pathogens have the capacity both to synthesize exogenous iNKT ligands and activate iNKT effector functions via the indirect mode. Recent work has begun to compare the contribution of both modes to pathogen-specific iNKT cell activation in vivo (Brigl et al., 2011). Fungi could fall in this latter category since they contain lipids that could potentially activate iNKT cells via CD1d. However, Brenner and colleagues tested *Aspergillus* lipid extracts for iNKT stimulatory activity and found none. Instead, the stimulatory activity resided in the heat-, acid-, and base-resistant fraction that is highly enriched in β-glucan polymers. Furthermore, dendritic cells (DCs) deficient in dectin-1, a C-type lectin receptor that binds β-(1,3) glucans (Drummond and Brown, 2011), showed defective iNKT cell activation following stimulation with *A. fumigatus* as well as a range of other clinically important fungal pathogens (*Candida albicans*, *Histoplasma capsulatum*, and *Alternaria alternata*). Although dectin-1 can mediate iNKT cell activation following fungal exposure, redundant activation mechanisms (via Toll-like and other C-type lectin receptors) ensure that iNKT cell activation occurs even with fungal pathogens (e.g., *Cryptococcus neoformans*) that do not expose dectin-1 ligands on their cell surface (Kawakami et al., 2001).

β-(1,3) glucans confer rigidity and strength to the fungal cell wall and are conserved across many different fungi. Obligate β-glucan exposure occurs during growth (Figure 1), for example as *A. fumigatus* conidia undergo swelling, the first step in germination into filamentous hyphae, or as *C. albicans* divides, resulting in bud and birth scars. From a fungal perspective, tight regulation of

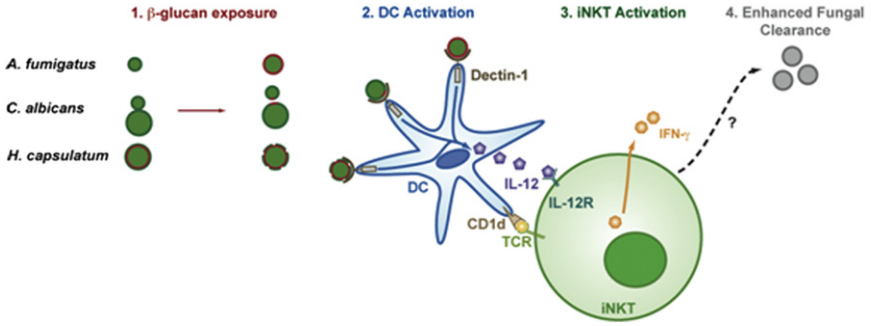

Figure 1. Model of Fungal iNKT Activation

In (1), fungi expose cell wall β-(1,3) glucan following morphological transitions (e.g., conidial swelling [*Aspergillus fumigatus*]), cell division (e.g., bud or birth scars [*Candida albicans*]), or disruption of the external α-glucan layer (*Histoplasma capsulatum*). In (2), β-(1,3) glucan ligation at the cell surface or within phagosomes induces dectin-1 signaling, which, in concert with collaborative Toll-like receptor signaling, leads to DC activation. In (3), DC-derived IL-12 is a key element for iNKT activation together with CD1d-dependent TCR interactions, although *A. fumigatus*-derived lipids do not appear to contribute to iNKT activation, consistent with a role for endogenous lipid antigen (shown in yellow) presentation to the iNKT TCR. In (4), rapid release of effector cytokines such as IFN-γ by iNKT cells follows activation. How iNKT activation mediates enhanced fungal clearance in vivo remains an important unresolved question.

β-glucan exposure likely confers survival benefits in natural environments, while mammalian β-glucan detection acts as a fungal growth indicator, inducing targeted responses that include iNKT cell activation.

Although Cohen et al. do not find a role for *A. fumigatus* lipids in direct iNKT activation, dendritic cell CD1d expression remains essential. This suggests that CD1d presents endogenous lipids during fungus-induced iNKT cell activation. It is unclear if β-(1,3) glucan recognition impacts the nature and agonist properties of endogenous lipids during this process. A recent study by Brennan et al. provides evidence for such a lipid repertoire shift in DCs following TLR stimulation, resulting in the CD1d-dependent presentation of an endogenous lipid with potent agonist properties (β-D-glucopyranosylceramide) (Brennan et al., 2011).

Cohen et al. show that IL-12 is dispensable for iNKT cell airway accumulation during respiratory fungal infection, but essential for IFN-γ production. The role of other cytokines and chemokines in this process remains poorly understood, but a previous study implicates MCP-1/CCL2 in iNKT cell accumulation in a respiratory cryptococcal infection model (Kawakami et al., 2001), and further experiments are required to dissect the molecular cues that mediate iNKT recruitment and activation. The lung-cell population that activates iNKTs in situ has not been identified; both alveolar macrophages and lung monocyte-derived $CD11b^+$ dendritic cells take up *A. fumigatus* spores in vivo (Hohl et al., 2009). Finally, how iNKT cell activation is coupled to rapid fungal clearance remains unclear as well (Figure 1), but does not appear to involve iNKT IL-17 production. It will be interesting to see whether other innate-like T lymphocytes such as the recently characterized mucosal-associated invariant T cells (MAITs) respond to fungal infections in a similar fashion.

How iNKT cells contribute to the pathogenesis of fungus-induced allergenic or invasive disease states represents an important area of future research. In patients with invasive aspergillosis (IA), loss of neutrophil number or function represents a significant clinical risk that may be diminished by auxiliary host defenses. For example, NK cells confer protective benefits in murine models of neutropenic aspergillosis (Park et al., 2009). Whether iNKT cells can enhance fungal clearance in the context of host immune damage remains undefined. Exploring the population dynamics and mechanisms of iNKT activity during fungal disease states is likely to inform new opportunities for therapeutic intervention.

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